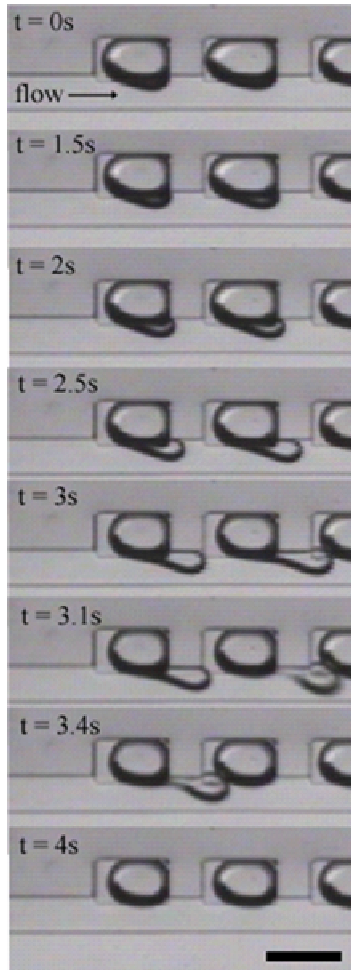# Supplementary Information
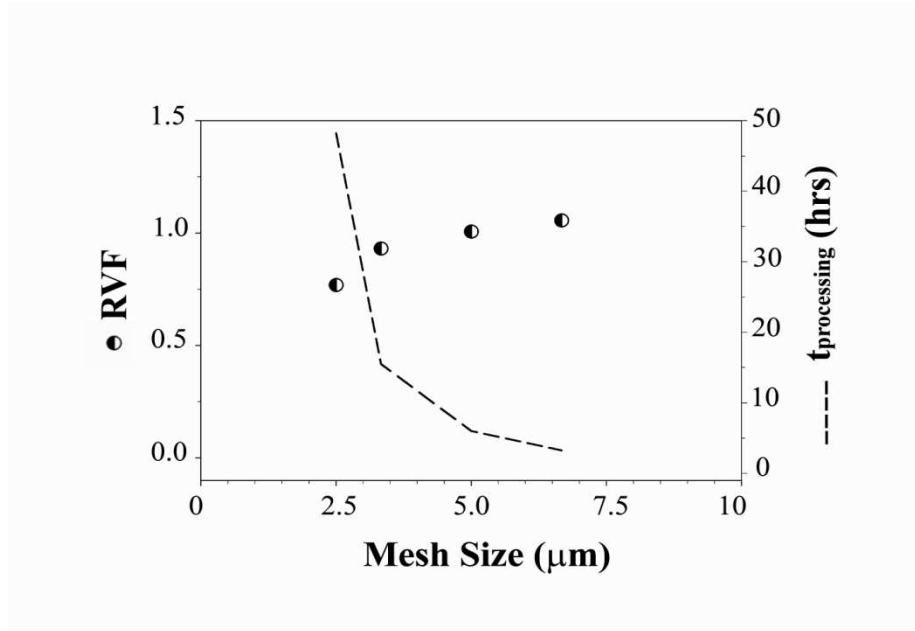

Supplementary Figure 1. Digitized samples could be released from the sample compartments by flowing immiscible phase through the device at an elevated flow rate. In these images, the sample phase was 1nM phosphate buffer and the immiscible phase was light mineral oil with 0.01% Span 80. The flow rate of the immiscible phase was 3μL per minute. The scale bar corresponds to 200 μm.
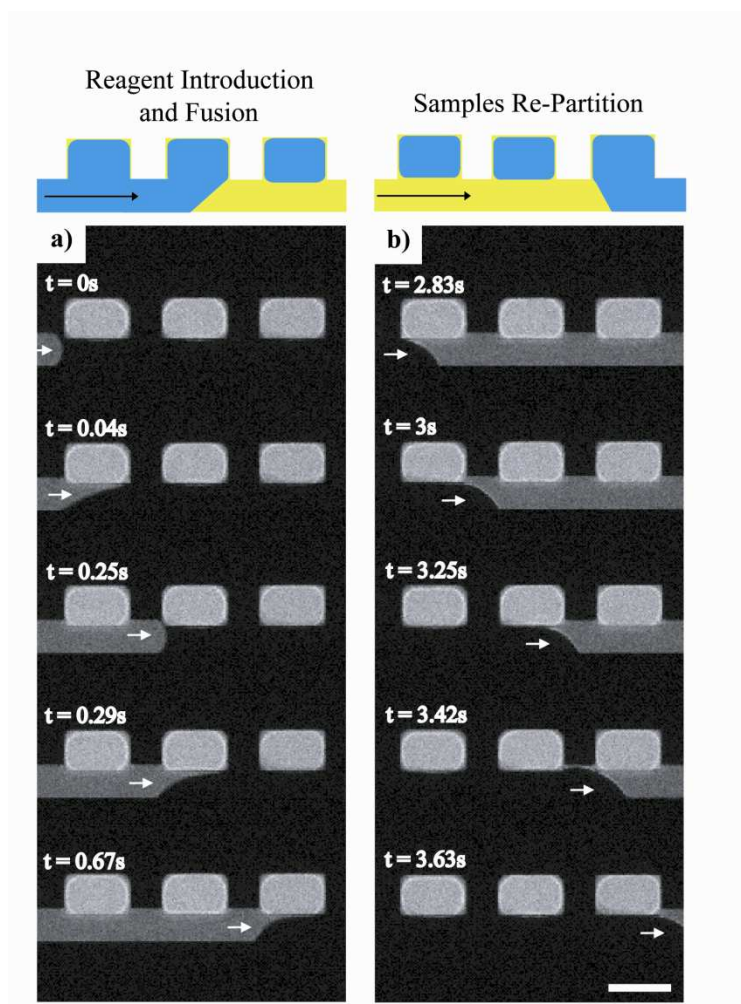
Supplementary Figure 2. Aqueous reagents could be added to digitized samples by flowing an aqueous reagent through the main channel and displacing the immiscible phase. Blue in schematic denotes aqueous phase (sample and additional reagent) and yellow denotes immiscible phase. An experimental time sequence illustrates this phenomenon (**a**) and **b**)). The images show the fluorescence of the sample phase and the aqueous reagent (both were 100μM Alexa488). The immiscible phase was light mineral oil with 0.01% Span 80. **a**) As immiscible phase was displaced from the main channel by the reagent phase, the aqueous reagent fused with aqueous samples in the side cavities. Fusion is seen in the images by an abrupt change in the shape of the

aqueous/immiscible phase interface. For example, see the first two sequential images in the left-hand panel, t = 0s and t = 0.04s. **b**) Following fusion, the digitized samples were re-partitioned by flowing immiscible phase through the main channel. If the aqueous plug in the main channel is long and remains fused with multiple digitized volumes along the main channel, there is risk of diffusional cross mixing between the digitized sample volumes. To minimize this issue, a short reagent plug should be used. The scale bar corresponds to 200 µm.

Supplementary Figure 3. The effect of changes in mesh size on the final RVF of the digitized sample (Vdrop/Vchamber) and simulation run time. The dashed line shows the change in the required simulation run time versus the mesh size. A 3-fold decrease in mesh size (i.e., $\delta = 6.67$ μm to $\delta = 2.5$ μm, or 4,712 nodes to 74,225 nodes) resulted in a reduction in the digitized sample volume by 27.2%, and an increase in simulation run time from 3.25 hours to between 48 and 80 hours.

S4

d₁ d₂ d₃ d₄ h₂ h₁
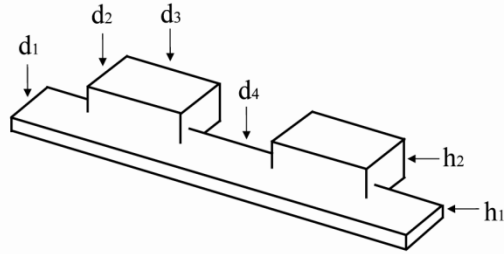
d2	d3	h2	Area of Cavity Opening	Surface Area of Cavity	Area of Opening/Area of Channel	Surface Area of Cavity/Area of Channel	Fraction of Cavities Filled	Number of Trials
30	300	110	12000	90600	3	22.65	0	13
300	300	110	12000	312000	3	78	1	30
125	200	110	8000	121500	2	30.375	1	38
20	20	110	800	9600	0.2	2.4	0	96
200	200	114	8000	171200	2	42.8	1	19
300	200	114	8000	234000	2	58.5	1	19
400	200	114	8000	296800	2	74.2	1	19
500	200	114	8000	359600	2	89.9	1	19
125	300	113	12000	171050	3	42.7625	1	15
125	400	113	16000	218650	4	54.6625	1	11
125	500	113	20000	266250	5	66.5625	1	10
300	300	113	12000	315600	3	78.9	1	15
30	25	75	1000	9750	0.25	2.4375	0	46
50	25	75	1000	13750	0.25	3.4375	0	45
30	50	75	2000	15000	0.5	3.75	0	76
125	100	75	4000	58750	1	14.6875	0.6	28
125	50	75	2000	38750	0.5	9.6875	0.026	39
62.5	100	75	4000	36875	1	9.21875	0.214	28
62.5	50	75	2000	23125	0.5	5.78125	0	39
125	200	80	8000	102000	2	25.5	1	97
30	300	80	12000	70800	3	17.7	0	13
300	300	80	12000	276000	3	69	1	30
125	200	80	8000	102000	2	25.5	1	38
20	20	80	800	7200	0.2	1.8	0	96
200	200	80	8000	144000	2	36	1	19
300	200	80	8000	200000	2	50	1	19
400	200	80	8000	256000	2	64	1	19
500	200	80	8000	312000	2	78	1	19
125	300	74	12000	137900	3	34.475	1	15
125	400	74	16000	177700	4	44.425	1	11
125	500	74	20000	217500	5	54.375	1	10
300	300	74	12000	268800	3	67.2	1	15
30	25	113	1000	13930	0.25	3.4825	0	46
50	25	113	1000	19450	0.25	4.8625	0	45
30	50	113	2000	21080	0.5	5.27	0	76
125	100	116	4000	77200	1	19.3	0.804	28
125	50	116	2000	53100	0.5	13.275	0	39
62.5	100	116	4000	50200	1	12.55	0.679	28
62.5	50	116	2000	32350	0.5	8.0875	0	39
125	200	122	8000	129300	2	32.325	1	97

Supplementary Figure 4. List of all geometric variants of the side chambers in which sample digitization was evaluated. h_1 and d_1 were held constant at 40μm and 100μm, respectively. The fraction of filled cavities is listed for each iteration.